underlying disease. We reported a girl, with a clinical diagnosis of sporadic NF1 during childhood, who presented a glioblastoma at 16 years-old. Faced with the NF1-like phenotype, diagnosis of CMMRD was suspected because tumor was ultra-hypermutated (228.67 mut/Mb), with a loss of PMS2 expression in both tumor and normal cells. Germline analyses identified a compound heterozygous pathogenic variant (PV) in the PMS2 gene, with an abnormal methylation tolerance test, that confirmed CMMRD. Moreover, a NF1 PV (20% and 9% in blood and saliva samples respectively) was identified compatible with a germline mosaicism. Patient's phenotype was atypical for CMMRD, with a voluminous neurofibroma and ephelids rather observed in NF1. CMMRD oncogenesis is not currently understood, in particular involvement of an NF1 PV, which could arise early from the ultra-hypermutated burden and might explain clinical signs, in particular CALMs. CMMRD diagnosis allowed proposing an adapted genetic counseling and surveillance for the patient and her parents according to the published guidelines due to the major impact on the patient's oncological risks and prognosis. The best strategy for surveillance of the neurofibroma is still debated due to uncertainties about its risk of degeneration with a CMMRD underlying disease. This observation raises the question of the frequency of mosaic NF1 germline PV in CMMRD-patients and the time of its postzygotic appearance in the context of a biallelic deficit of one of the MMR genes. Combination of these both CMMRD and NF1 germline PVs would be a strong argument for a combination of MEK-inhibitors with immunotherapy.

HGG-41. GLIOMA ONCOGENESIS IN THE CONSTITUTIONAL MISMATCH REPAIR DEFICIENCY (CMMRD) SYNDROME

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PURPOSE: Constitutional Mismatch Repair Deficiency (CMMRD) is a cancer predisposition due to bi-allelic mutations in one of the four main mismatch repair (MMR) genes (PMS2, MSH2, MSH6 or MLH1) associated with early onset of cancers, especially glioblastomas (GBM). Our aim was to decipher the molecular specificities of gliomas occurring in this context. METHODS: A comprehensive analysis of clinical, histopathological and genomic data (whole exome sequencing) was performed for 12 children with a CMMRD for which we had available frozen brain tumor material (10 GBM and 2 anaplastic astrocytomas). RESULTS: Eight patients harbored an ultra-mutated phenotype with more than 100 somatic non synonymous (NS) SNV/Mb. No correlation was observed between the number of mutation and sex, age, overall survival or mutated MMR gene. POLE and POLD1 exonuclease domain driver somatic mutations were described for eight and one patients respectively. The 4/12 tumors without POLE somatic mutation did not show the classical ultra-hypermutation pattern. All patients with POLE mutation had already more than 20 NS SNV/Mb (median 40, [range 23-114]) suggesting that the hypermutation phenomenon started before the appearance of the somatic POLE mutation. The mutational signatures of the tumors, dominated by the MMR signatures, were not modified after the onset of the POLE mutation when analyzing the different mutation bursts. Specific recurrent somatic mutations were observed in SETD2 (9/12), TP53 (9/12), NF1 (9/12), EPHB2 (8/12), and DICER1 (7/12). Only half of the tumors overexpressed PDL1 by immunohistochemistry and this overexpression was not associated with a higher tumor mutation burden. CONCLUSION: CMMRD-associated gliomas have a specific oncogenesis that does not trigger usual pathways and mutations seen in sporadic pediatric or adult GBM. Frequent alterations in other pathways (e.g. MAPK or DNA-PK pathway) may suggests the use of other targeted therapies aside from PD1 inhibitors.

HGG-42. EVOLUTIONARY SELECTION OF KEY ONCOGENIC ALTERATIONS IN PATIENT-DERIVED MODELS OF PAEDIATRIC DIFFUSE HIGH GRADE GLIOMA (PDHGG) SUBTYPES *IN VITRO* AND *IN VIVO*

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PDHGG are a diverse group of childhood brain tumours comprising multiple subgroups carrying distinct molecular drivers. Patient-derived models accurately recapitulating this underlying biology are critical for mechanistic/preclinical studies aimed at improving patient outcome, however their behaviour over time in the environments in which they are propagated, and how this relates to the human disease, is largely unknown. To explore this, we collected 94 models of PDHGG established as 2D/3D stem cell cultures in vitro, and generated patient-derived xenografts (PDX) in 33/62 specimens implanted orthotopically in vivo. We carried out exome/targeted sequencing, methylation profiling and RNAseq to profile cells through their first 25 passages in culture, and sequential implantation from p0-p2 in mice. In 15/83 cultures, we observed enrichment of gene expression signatures of non-malignant cells over the first 5 passages, with concurrent depletion of somatic mutations/CNAs, excluding them from further study. Validated models retained tumour-matched genotypes, CNAs and driver alterations including H3.3G34R, H3.3/H3.1K27M, BRAF and ACVR1 over time, however subclonal alterations underwent selection in culture which profoundly altered their response to targeted drug treatment. In 6/7 PDGFRA-mutant models, activating mutations were selected against between p5-20 in 2D and/or 3D, whilst MAPK pathway mutations in *NF1/PIK3R1* similarly diverged over 15 passages under different growth conditions, resulting in isogenic models with differential signalling, in vivo tumorigenicity, and in vitro sensitivity to multiple MEK inhibitors. In PDXs, serial xenografting reduced the time to tumour formation by up to half, with a concomitant shift in clonal architecture. Multi-region sequencing of diffusely-infiltrating tumours showed selection for alterations such as PIK3CA/NF1 at distant sites, with evidence for convergent evolution of subclonal mutations, as in human tumours. Understanding the evolutionary dynamics of targetable/predictive alterations in PDHGG model systems is key to developing new and effective therapeutic interventions in this highly heterogenous disease.

HGG-43. ABROGATION OF EXOSOME BIOGENESIS SIGNIFICANTLY AFFECTS CELL MOTILITY IN HETEROGENOUS SUB-POPULATIONS OF PAEDIATRIC-TYPE DIFFUSE HIGH-GRADE GLIOMA

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Paediatric-type diffuse High-Grade Gliomas (PDHGG) are highly heterogenous tumours comprised of distinct cell sub-populations co-existing within the same tumour mass. We have shown that primary patient-derived sub-clones, as well as optical-barcoded sub-clones, function as an interconnected network conferring an aggressive phenotype. Here, we explored the role of exosomes in mediating PDHGG inter-clonal communication. A comprehensive characterization of 7 optical-barcoded single cell-derived clones obtained from two patient-derived cell lines (one DMGH3K27-altered and one diffuse high-grade paediatric-type glioma H3WT), confirmed extensive genomic and phenotypic heterogeneity. Live single-cell tracking in 3D migration and invasion assays demonstrated the key role of the inter-clonal crosstalk in driving a more aggressive phenotype. To determine the exosome role in this crosstalk, we first characterised them in terms of size, marker expression and cargo. Moreover, we demonstrated that exosomes were actively internalized by the sub-clones. Exosomal proteomic analysis showed differential protein contents implicated in the regulation of biological processes such as focal adhesion and extracellular matrix organization. The analysis of exosomal miRNome did not show differentially expressed miRNAs between sub-clones, however, specific and distinct exosomal miRNAs were found uniquely expressed by each sub-clone. The abrogation of the exosome biogenesis by GW4869 phospholipase inhibitor did not affect sub-clones viability, but significantly inhibited their motility, when cultured individually and more prominently in co-culture condition. Analysis of the exo-miRNAs uniquely expressed by the sub-clones highlighted a set of target genes regulating cell motility/invasion/migration. These target genes were differentially expressed when sub-clones were co-cultured compared to mono-culture.